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(54) Title: SECRETED PROTEINS AND NUCLEIC ACIDS ENCODING THEM

(57) Abstract

The invention provides isolated nucleic acid molecules, designated TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224 and TANGO 239. These nucleic acid molecules encode wholly secreted and transmembrane proteins. The invention also provides antisense nucleic acid molecules, expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a nucleic acid molecule of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and antibodies. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

```
                                                                              48
gga ttt gta ccg gag tcc cat ttg gga gca aga gcc atc tac tcg tcc
Gly Phe Val Pro Glu Ser His Leu Gly Ala Arg Ala Ile Tyr Ser Ser
 1               5                   10                  15

                                                                              96
gtt acc ggc ctt acc atg gat tgc caa gaa aat gag tac tgg gac
Val Thr Gly Leu Pro Thr Met Asp Cys Gln Glu Asn Glu Tyr Trp Asp
            20                  25                  30

                                                                             144
caa tgg gga cgg gtc tgt acc tgc caa cgg tgt cct gga cag gag
Gln Trp Gly Arg Val Cys Thr Cys Gln Arg Cys Pro Gly Gln Glu
            35                  40                  45

                                                                             192
cta tcc aag gat tgt ggt tat gga gga gac gat gcc tac tgc aca
Leu Ser Lys Asp Cys Gly Tyr Gly Gly Asp Asp Ala Tyr Cys Thr
            50                  55                  60

                                                                             240
gcc tgc cct cgc agg tac aaa agc agc tgg ggc cac cac aaa tgt
Ala Cys Pro Arg Arg Tyr Lys Ser Ser Trp Gly His His Lys Cys
65                  70                  75                  80

                                                                             288
cag agt atc acc tgt gct gtc atc aat cgt gtt cag aag gtc aac
Gln Ser Ile Thr Cys Ala Val Ile Asn Arg Val Gln Lys Val Asn
            85                  90                  95

                                                                             336
tgc aca gct acc tct aat gct tgt ggg gac tgt ttg ccc agg ttc
Cys Thr Ala Thr Ser Asn Ala Cys Gly Asp Cys Leu Pro Arg Phe
            100                 105                 110

                                                                             384
tac cga aag aca cgc att gga ggc ctg caa gac caa gag tgc atc ccg
Tyr Arg Lys Thr Arg Ile Gly Gly Leu Gln Asp Gln Glu Cys Ile Pro
            115                 120                 125

                                                                             432
tgc acg aag cag acc ccc acc tct gag gtt caa tgt gcc ttc ttg
Cys Thr Lys Gln Thr Pro Thr Ser Glu Val Gln Cys Ala Phe Leu
130                 135                 140
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Fig. 3

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agc tta gtg gag gca gat gca ccc aca gtg ccc cct cag gag gcc aca    480
Ser Leu Val Glu Ala Asp Ala Pro Thr Val Pro Pro Gln Glu Ala Thr
145                 150                 155                 160

ctt gtt gca ctg ttg cag gag gtt tgc tgc tgt agt ttg ata aaa       528
Leu Val Ala Leu Leu Gln Glu Val Cys Cys Val Ser Leu Ile Lys
                165                 170                 175

cag caa agg agg aat ctc tct tcc ccg tgc cac cca gca agg aga cca    576
Gln Gln Arg Arg Asn Leu Ser Ser Pro Cys His Pro Ala Arg Arg Pro
            180                 185                 190

gtg ctg agt ccc aag tgagtgagaa catctttcag accagccac ttaaccctat    631
Val Leu Ser Pro Lys
            195

cctcgaggac gactgcagct cgactagtgg cttccccaca caggagtcct ttaccatggc   691
ctcctgcacc tcagagagcc actcccactg gtccacagc  cccatcgaat gcacagagct   751
ggacctgcaa aagttttcca gctctgcctc ctatactgga gctgagacct tgggggaaa    811
cacagtcgaa agcactggag acaggctgga gctcaatgtg ccctttgaag ttcccagccc   871
ttaactctaa tgaggtctct tgggcccctg gcagccttgc ccagttgttc tctctggact   931
ctgttcctat accacaacag cagcaggggc ctgaaatgtg atgtccacaa gagctaatac   991
cctacagatg gggcatatcc tatcccatcc caccagagga ttgattctcc atttcacaag  1051
gactgatctg gagcacttct tgcttccctg ttgtagtctg gggagccaga ttccacattc  1111
atgggactac cagacatgtt cctagctcaa cttgattata gagaagagga gagaggacag  1171
tgaatggggt agggttttca tgtctgcatt tttggtcagg taagcctctc aaaattgtgt  1231
tggcacatct acctagcact ttagggacaa aatcaaaccc ttctcccctt ttagctcctc  1291
cacactgcct ccctcctcaa cacacacaca cacacataca catagacaca              1351
caaacacaca aatatctatc ttggggggag cctcgtgcca taattcccaa              1411
gttcatgtct cattgcagca tttgaggttc cctcgtgcca caaacacttt ccctctagat  1471
ccctgggggc tcaccctgta tttgaggttc tcaccaccct cagcaggag  aaggctgaa   1531
gttcgccatt ttgaaccctt acagaacatt tctgagccaa agtaatcttc cttctgggggc 1591
ctgagttccc caaactaccc cacagcagtc cctcaaagac agccctcaat ccatgtaggg  1651
acatctgagt atgctctctt ctattgaaat gtcaattcaa tcccagcttt ctcaccaccg  1711
ttcccctttg attctttctc aattgtcttt ttgcctttag ctcccaccta tacatctcat  1771
gctcagagaa aaacaagttc cttagaggtt gtattcttta ttctccaaga atctgtctga  1831
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Fig. 3A

```
1891  aacttgtaca gctagttcct tattaagtgg tttattaagt acattaggca
1951  gaatgtgcac ttcatcacca ctggcaaagg agtgctgtct acagcaagat
2011  tttgctttt  agaattttat tttggttc   atccatctac aaacactgat
2071  taaggcccc  tggggcaacc attactaaaa ggacttggga aaaagcaaaa
2131  aggtcccatt gtactggact agcaattgaa atacaagcct gtaccaagca
2191  agcagcctgg cccacacag  gtattagcaa accaagtt   taggccttgg
2251  cctttaggtt tcctgtttt  ttttgtgtt  tgttttttc  tgcaacaggt
2311  tattcttatc tcactggctt tgtttagacc ttctggtaga agaaataata
2371  tccagacagg ggatgatttg gcttcagcag ttcaaaggtt gccatgtggc
2431  tggcagtggt tcaagcccac atttgacact aggaaagata atgatggtaa
2491  cacagtaata ataataataa taacaaaaat gaaagagtaa gacttcttca
2551  gtgtgcttgc tcatggcatg aatgctatgt agcagccca  agaatcacct
2611  taattccaac tttttgaggt tcagcaattg gaggtggcaa atttaaagt
2671  atttcgggta aaggtgaagt cgtcttata  ttggctttgc ggccatggca
2731  aataccatag ttgagtattt agttctttt  acagttttac ttttcaatgc
2791  tgaggcatat ttctttgagc tatgtgcttt tctacaaagg ggttattggt
2851  cagtggaaga acaaagtaca cttgataaaa catacattga gcctaaacag
2911  cagttaagtt gtctctaaat aaaaaaaaaa tgtagttttt gtttgtaagg
2971  aagggaggt  aatgaattt  tttttttt   ggattactgt tttttctctcc
3031  atataccttg atttcctgag gagtctggga aaataatttt ttcctccaag
3091  attctcagat acttggattt aaaggattca gcactacagc acaacataca
3151  gccctgtcac ccaggttagg taatccctcc tgtcccactc acccccaccct
3211  actagctagg attgagatca tttacattga atattggtac tttttctca
3271  taaatgggtt tcttcagtgt atttatagag ctcttgagcc gtagatactg
3331  gcagggctat atttgtttat aacacatttca tattggccaa taaacagaaa
3385  tatatccaaa aaaaaaaaaa aaaaaaaaaa aaaggggcgg ccgc
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Fig. 3B

acid residues 147 to 170 of SEQ ID NO:5 (SEQ ID NO:), and a cytoplasmic domain at amino acid residues 171 to 206 of SEQ ID NO:5 (SEQ ID NO:).

The extracellular region of human TANGO 140-1 includes TNF-R domains from about amino acids 11 to 49 of SEQ ID NO:5 (SEQ ID NO:100) and from about amino acids
5   52-91 of SEQ ID NO:5 (SEQ ID NO:101).

A clone, EpDH137, which encodes human TANGO 140-1 was deposited as part of EpDHMix1 with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209) on November 20, 1998 which was assigned Accession Number 98999. This deposit will be maintained under the terms of the Budapest Treaty on the
10 International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Figure 10 depicts a hydropathy plot of human TANGO 140-1. Relatively hydrophobic residues are above the horizontal line, and relatively hydrophilic residues are
15 below the horizontal line. As shown in the hydropathy plot, amino acids 147 to 170 of SEQ ID NO:5 (SEQ ID NO:36) correspond to a transmembrane domain of TANGO 140-1. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace.

20 HUMAN TANGO 140-2

An additional clone having significant homology to human TANGO 140-1 was identified. The clone was sequenced and is likely to be a splice variant of TANGO 140-1. This variant is referred to herein as TANGO 140-2. The human TANGO 140-2 includes a 3385 nucleotide cDNA (Figure 3; SEQ ID NO:6). It is noted that the nucleotide sequence
25 depicted in SEQ ID NO:6 contains *a Not I* adapter sequence on the 3' end (5' GGGCGG CCGC 3' (SEQ ID NO:)). Thus, it is to be understood that the nucleic acid molecules of the invention include not only those sequences with such adaptor sequences but also the nucleic acid sequences described herein lacking the adaptor sequences. The open reading frame of TANGO 140-2 comprises nucleotides 2 to 619 of SEQ ID NO:6 (SEQ ID NO:38), and
30 encodes a 198 amino acid putative secreted protein (Figure 3; SEQ ID NO:7).

Human TANGO 140-2 also includes TNF-R domains from about amino acids 25 to 63 of SEQ ID NO:7 (SEQ ID NO:102), and from about amino acids 66 to 105 of SEQ ID NO:7 (SEQ ID NO:103).

A clone, EpDH185, which encodes human TANGO 140-2 was deposited as part of
35 EpDHMix1 with the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209) on November 20, 1998 which was assigned Accession Number

98999. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

5   Figure 11 depicts a hydropathy plot of TANGO 140-2. Relatively hydrophobic residues are above the horizontal line, and relatively hydrophilic residues are below the horizontal line. As shown in the hydropathy plot, TANGO 140-2 does not have a transmembrane domain. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace.

10

Uses of TANGO 140 Nucleic Acids, Polypeptides, and Modulators Thereof

        The TANGO 140 proteins of the invention comprise a family of proteins having sequence similarity to members of the TNF-R superfamily. Thus, the TANGO 140 proteins of the invention are members of the TNF-R superfamily. Accordingly, TANGO 140

15 proteins likely function in a similar manner as members of the TNF-R family and TANGO 140 modulators can be used to treat any TNF-R/NGF-R-associated disorders.

        For example, members of the tumor necrosis factor receptor (TNF-R) superfamily regulate a diverse range of cellular processes including cell proliferation, programmed cell death and immune responses. TNF-R family members are cell surface proteins which

20 function as receptors for cytokines. Mallet et al. (1991) *Immunology Today* 12:220-223. For example, the binding of NGF to NGF-R causes neuronal differentiation and survival. Barde (1989) *Neuron* 2:1525-1534. Similarly, the TANGO 140 molecules of the invention can modulate neuronal differentiation and survival.

        NGF (nerve growth factor) induces, *inter alia,* neurite outgrowth and promotes

25 survival of embryonic sensory and sympathetic neurons. Nerve growth factor (NGF) is also involved in the development and maintenance of the nervous system. Thus, TANGO 140 polypeptides, nucleic acids and/or modulators thereof can be used to modulate the function, morphology, proliferation and/or differentiation of cells in the nervous system. Such molecules may be used in the treatment of neural disorders, including, without limitation,

30 epilepsy, muscular dystrophy, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease).

        In addition, both TGF-α and TGF-β bind to TGF-RI and TGF-RII, leading to a diverse range of effects including inflammation and tumor cell death. Beutler et al. (1989) *Ann. Rev. Immunol.* 7:625-655; Sprang (1990) *Trends Biochem. Sci.* 15:366-368. Thus, the

35 TANGO 140 proteins of the invention are likely to bind directly or indirectly to a soluble

used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook et al., *supra*.

5   In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-
10   275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-
15   specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the á-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

20   The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to the mRNA encoding a polypeptide of the invention. Regulatory sequences
25   operably linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or
30   attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub et al. (*Reviews - Trends in Genetics*, Vol. 1(1) 1986).

Another aspect of the invention pertains to host cells into which a recombinant
35   expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms

refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

5   A host cell can be any prokaryotic (e.g., *E. coli*) or eukaryotic cell (e.g., insect cells, yeast or mammalian cells).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing 10 foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the 15 expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and 20 methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

In another embodiment, the expression characteristics of an endogenous (*e.g.*, TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224, and 25 TANGO 239) nucleic acid within a cell, cell line or microorganism may be modified by inserting a DNA regulatory element heterologous to the endogenous gene of interest into the genome of a cell, stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous gene (*e.g.*, TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224, and TANGO 239) 30 and controls, modulates or activates the endogenous gene. For example, endogenous TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224, and TANGO 239 which are normally "transcriptionally silent", *i.e.*, TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224, and TANGO 239 genes which are normally not expressed, or are expressed only at very low levels in a cell line or 35 microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or

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microorganism. Alternatively, transcriptionally silent, endogenous TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224, and TANGO 239 genes may be activated by insertion of a promiscuous regulatory element that works across cell types.

5   A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with and activates expression of endogenous TANGO 128, TANGO 140, TANGO 197, TANGO 212, TANGO 213, TANGO 224, and TANGO 239 genes, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May
10   16, 1991.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce a polypeptide of the invention. Accordingly, the invention further provides methods for producing a polypeptide of the invention using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention
15   (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another embodiment, the method further comprises isolating the polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic
20   animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which a sequences encoding a polypeptide of the invention have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous sequences encoding a polypeptide of the invention have been introduced into their genome or homologous recombinant animals in which endogenous
25   encoding a polypeptide of the invention sequences have been altered. Such animals are useful for studying the function and/or activity of the polypeptide and for identifying and/or evaluating modulators of polypeptide activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of
30   transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, an "homologous recombinant
35   animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the

endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing nucleic acid encoding a polypeptide of the invention (or a homologue thereof) into the male pronuclei of
5   a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the transgene to direct expression of the polypeptide of the invention to particular cells. Methods for
10 generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent NOS. 4,736,866 and 4,870,009, U.S. Patent No. 4,873,191 and in Hogan, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals.
15 A transgenic founder animal can be identified based upon the presence of the transgene in its genome and/or expression of mRNA encoding the transgene in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying the transgene can further be bred to other transgenic animals carrying other transgenes.
20   To create an homologous recombinant animal, a vector is prepared which contains at least a portion of a gene encoding a polypeptide of the invention into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the gene. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous gene is functionally disrupted (i.e., no longer encodes a
25 functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous protein). In the homologous recombination vector, the altered portion of the gene is flanked at its 5' and 3'
30 ends by additional nucleic acid of the gene to allow for homologous recombination to occur between the exogenous gene carried by the vector and an endogenous gene in an embryonic stem cell. The additional flanking nucleic acid sequences are of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (*see, e.g.,*
35 Thomas and Capecchi (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation)

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and cells in which the introduced gene has homologously recombined with the endogenous gene are selected (*see, e.g.,* Li et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (*see, e.g.,* Bradley in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach,*
5   Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous
10 recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Current Opinion in Bio/Technology* 2:823-829 and in PCT Publication NOS. WO 90/11354, WO 91/01140, WO 92/0968, and WO 93/04169.

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One
15 example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, *see, e.g.,* Lakso et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the
20 transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced
25 according to the methods described in Wilmut et al. (1997) *Nature* 385:810-813 and PCT Publication NOS. WO 97/07668 and WO 97/07669.

IV. Pharmaceutical Compositions

The nucleic acid molecules, polypeptides, and antibodies (also referred to herein as
30 "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and
35 absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in

the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

5   The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a polypeptide or nucleic acid of the invention. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid of the invention. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a

10   pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid of the invention and one or more addtional active compounds.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal

15   (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating

20   agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

25   Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be

30   sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable

35   mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of

dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol,

5   sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with one

10   or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and

15   freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and

20   used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can

25   contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl

30   salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For

35   transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and

include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

5   The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release 10 formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal 15 suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage 20 unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and 25 directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

For antibodies, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 30 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is 35 described by Cruikshank et al. ((1997) *J. Acquired Immune Deficiency Syndromes and . Human Retrovirology* 14:193).

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. Patent 5,328,470) or by stereotactic injection (*see, e.g.,* Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The

5   pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

10   The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described

15   herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology); c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and d) methods of treatment (e.g., therapeutic and prophylactic). For example, polypeptides of the invention can to used to (i) modulate cellular proliferation; (ii)

20   modulate cell migration and chemotaxis; (iii) modulate cellular differentiation; and/or (iv) modulate angiogenesis. The isolated nucleic acid molecules of the invention can be used to express proteins (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect mRNA (e.g., in a biological sample) or a genetic lesion, and to modulate activity of a polypeptide of the invention. In addition, the polypeptides of the

25   invention can be used to screen drugs or compounds which modulate activity or expression of a polypeptide of the invention as well as to treat disorders characterized by insufficient or excessive production of a protein of the invention or production of a form of a protein of the invention which has decreased or aberrant activity compared to the wild type protein. In addition, the antibodies of the invention can be used to detect and isolate a protein of the

30   and modulate activity of a protein of the invention.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

A.   Screening Assays

35   The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides,

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peptidomimetics, small molecules or other drugs) which bind to polypeptide of the invention or have a stimulatory or inhibitory effect on, for example, expression or activity of a polypeptide of the invention.

In one embodiment, the invention provides assays for screening candidate or test
5   compounds which bind to or modulate the activity of the membrane-bound form of a polypeptide of the invention or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods
10 requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

15   Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop et al. (1994)
20 *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Bio/Techniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent NOS. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al. (1992) *Proc. Natl. Acad. Sci.*
25 *USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici (1991) *J. Mol. Biol.* 222:301-310).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion
30 thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to the polypeptide determined. The cell, for example, can be a yeast cell or a cell of mammalian origin. Determining the ability of the test compound to bind to the polypeptide can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the polypeptide or
35 biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either

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directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In a
5   preferred embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test
10   compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind to the polypeptide or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of a polypeptide of the invention, or a biologically
15   active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the polypeptide or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of the polypeptide or a biologically active portion thereof can be accomplished, for example, by determining the ability of the polypeptide protein to bind to
20   or interact with a target molecule.

Determining the ability of a polypeptide of the invention to bind to or interact with a target molecule can be accomplished by one of the methods described above for determining direct binding. As used herein, a "target molecule" is a molecule with which a selected polypeptide (e.g., a polypeptide of the invention binds or interacts with in nature,
25   for example, a molecule on the surface of a cell which expresses the selected protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A target molecule can be a polypeptide of the invention or some other polypeptide or protein. For example, a target molecule can be a component of a signal transduction pathway which
30   facilitates transduction of an extracellular signal (e.g., a signal generated by binding of a compound to a polypeptide of the invention) through the cell membrane and into the cell or a second intercellular protein which has catalytic activity or a protein which facilitates the association of downstream signaling molecules with a polypeptide of the invention. Determining the ability of a polypeptide of the invention to bind to or interact with a target
35   molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a

cellular second messenger of the target (e.g., intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to a polypeptide of the invention operably linked to a nucleic acid encoding a detectable marker, e.g.

5   luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a polypeptide of the invention or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the

10   polypeptide or biologically active portion thereof. Binding of the test compound to the polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the polypeptide of the invention or biologically active portion thereof with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining

15   the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind to the polypeptide or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting a

20   polypeptide of the invention or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the polypeptide or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of the polypeptide can be accomplished, for example, by determining the ability of the polypeptide to bind to a target molecule by one

25   of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of the polypeptide can be accomplished by determining the ability of the polypeptide of the invention to further modulate the target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously

30   described.

In yet another embodiment, the cell-free assay comprises contacting a polypeptide of the invention or biologically active portion thereof with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein

35 · determining the ability of the test compound to interact with the polypeptide comprises .

determining the ability of the polypeptide to preferentially bind to or modulate the activity of a target molecule.

The cell-free assays of the present invention are amenable to use of both a soluble form or the membrane-bound form of a polypeptide of the invention. In the case of cell-free

5   assays comprising the membrane-bound form of the polypeptide, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the polypeptide is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-octylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton X-100, Triton X-114, Thesit,

10 Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention,

15 it may be desirable to immobilize either the polypeptide of the invention or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to the polypeptide, or interaction of the polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable

20 for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase fusion proteins or glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, MO) or glutathione

25 derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or A polypeptide of the invention, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is

30 measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of binding or activity of the polypeptide of the invention can be determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the polypeptide of the invention or

35 its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated polypeptide of the invention or target molecules can be prepared from biotin-

NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the polypeptide of the invention or target molecules but which do not interfere with binding of the polypeptide of

5   the invention to its target molecule can be derivatized to the wells of the plate, and unbound target or polypeptidede of the invention trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the polypeptide of the invention or target molecule, as well as enzyme-linked assays

10   which rely on detecting an enzymatic activity associated with the polypeptide of the invention or target molecule.

In another embodiment, modulators of expression of a polypeptide of the invention are identified in a method in which a cell is contacted with a candidate compound and the expression of the selected mRNA or protein (i.e., the mRNA or protein corresponding to a

15   polypeptide or nucleic acid of the invention) in the cell is determined. The level of expression of the selected mRNA or protein in the presence of the candidate compound is compared to the level of expression of the selected mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of expression of the polypeptide of the invention based on this comparison. For example,

20   when expression of the selected mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of the selected mRNA or protein expression. Alternatively, when expression of the selected mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the

25   candidate compound is identified as an inhibitor of the selected mRNA or protein expression. The level of the selected mRNA or protein expression in the cells can be determined by methods described herein.

In yet another aspect of the invention, a polypeptide of the inventions can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Patent No.

30   5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and PCT Publication No. WO 94/10300), to identify other proteins, which bind to or interact with the polypeptide of the invention and modulate activity of the polypeptide of the invention. Such binding proteins are also likely to be involved in the

35   propagation of signals by the polypeptide of the inventions as, for example, upstream or downstream elements of a signaling pathway involving the polypeptide of the invention.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B.   Detection Assays

5   Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic

10   identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

            Once the sequence (or a portion of the sequence) of a gene has been isolated, this

15   sequence can be used to map the location of the gene on a chromosome. Accordingly, nucleic acid molecules described herein or fragments thereof, can be used to map the location of the corresponding genes on a chromosome. The mapping of the sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

20   Briefly, genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the sequence of a gene of the invention. Computer analysis of the sequence of a gene of the invention can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids

25   containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the gene sequences will yield an amplified fragment. For a review of this technique, see D'Eustachio et al. ((1983) *Science* 220:919-924).

            PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day

30   using a single thermal cycler. Using the nucleic acid sequences of the invention to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a gene to its chromosome include *in situ* hybridization (described in Fan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-

35   selection by hybridization to chromosome specific cDNA libraries. Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further

be used to provide a precise chromosomal location in one step. For a review of this technique, see Verma et al. (Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York, 1988)).

5   Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

10   Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then

15   be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, e.g., Egeland et al. (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with a gene of the invention can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected

20   individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to

25   confirm the presence of a mutation and to distinguish mutations from polymorphisms.

In the instant case, the human gene for TANGO 128 was mapped on radiation hybrid panels to the long arm of chromosome 4, in the region q28-31. Flanking markers for this region are WI-3936 and AFMCO27ZB9. The FGC (fibrinogen gene cluster), GYP (glycophorin cluster), IL15 (interlukin 15), TDO2 (tryptophab oxygenase), and MLR

30   (mineralcorticoid receptor) genes also map to this region of the human chromosome. This region is syntenic to mouse chromosome 8. The Q (quinky), pdw (proportional dwarf), and lyl1 (lymphoblastomic leukemia) loci also map to this region of the mouse chromosome. Ill5 (interlukin 15), mlr (mineralcorticoid receptor), ucp (uncoupling protein), and clgn (calmegin) genes also map to this region of the mouse chromosome.

35   In the instant case, the human gene for TANGO 213 was mapped on radiation hybrid panels to the long arm of chromosome 17, in the region p13.3. Flanking markers for

this region are WI-5436 and WI-6584. The MDCR (Miller-Dieker syndrome), PEDF
(pigment epithelium derived factor), and PFN1(profillin 1) genes also map to this region of
the human chromosome. This region is syntenic to mouse chromosome 11, locus 46(g).
The ti (tipsy) loci also maps to this region of the mouse chromosome. The pfn1 (profilin 1),
5   htt (5-hydroxytryptamine (serotonin) transporter), acrb (acetylcholine receptor beta)
     genes also map to this region of the mouse chromosome.

2. Tissue Typing

          The nucleic acid sequences of the present invention can also be used to identify
10   individuals from minute biological samples. The United States military, for example, is
     considering the use of restriction fragment length polymorphism (RFLP) for identification
     of its personnel. In this technique, an individual's genomic DNA is digested with one or
     more restriction enzymes, and probed on a Southern blot to yield unique bands for
     identification. This method does not suffer from the current limitations of "Dog Tags"
15   which can be lost, switched, or stolen, making positive identification difficult. The
     sequences of the present invention are useful as additional DNA markers for RFLP
     (described in U.S. Patent 5,272,057).

          Furthermore, the sequences of the present invention can be used to provide an
     alternative technique which determines the actual base-by-base DNA sequence of selected
20   portions of an individual's genome. Thus, the nucleic acid sequences described herein can
     be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These
     primers can then be used to amplify an individual's DNA and subsequently sequence it.

          Panels of corresponding DNA sequences from individuals, prepared in this manner,
     can provide unique individual identifications, as each individual will have a unique set of
25   such DNA sequences due to allelic differences. The sequences of the present invention can
     be used to obtain such identification sequences from individuals and from tissue. The
     nucleic acid sequences of the invention uniquely represent portions of the human genome.
     Allelic variation occurs to some degree in the coding regions of these sequences, and to a
     greater degree in the noncoding regions. It is estimated that allelic variation between
30   individual humans occurs with a frequency of about once per each 500 bases. Each of the
     sequences described herein can, to some degree, be used as a standard against which DNA
     from an individual can be compared for identification purposes. Because greater numbers
     of polymorphisms occur in the noncoding regions, fewer sequences are necessary to
     differentiate individuals. The noncoding sequences of SEQ ID NO:1, 4, 6, 8, 11, 14, 17, 20,
35· 53, 56, 59, 62, 65, or 68 can comfortably provide positive individual identification with a
     panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of

100 bases. If predicted coding sequences, such as those in SEQ ID NO:3, 10, 13, 16, 19, 22, 38, 3955, 58, 61, 64, 67, 70, or 73, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

5   If a panel of reagents from the nucleic acid sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

10   3. Use of Partial Gene Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken

15   from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide

20   reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated

25   fragments. Sequences targeted to noncoding regions are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the nucleic acid sequences of the invention or portions thereof, e.g., fragments derived from noncoding regions having a length of at least 20 or 30 bases.

30   The nucleic acid sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such probes can be used to identify tissue by species and/or by organ type.

35

C.   Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically.

5   Accordingly, one aspect of the present invention relates to diagnostic assays for determining expression of a polypeptide or nucleic acid of the invention and/or activity of a polypeptide of the invention, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant expression or activity of a polypeptide of

10   the invention. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, mutations in a gene of the invention can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the

15   onset of a disorder characterized by or associated with aberrant expression or activity of a polypeptide of the invention.

Another aspect of the invention provides methods for expression of a nucleic acid or polypeptide of the invention or activity of a polypeptide of the invention in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to

20   herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual examined to determine the ability of the individual to respond to a particular agent).

Yet another aspect of the invention pertains to monitoring the influence of agents

25   (e.g., drugs or other compounds) on the expression or activity of a polypeptide of the invention in clinical trials. These and other agents are described in further detail in the following sections.

1.   Diagnostic Assays

30   An exemplary method for detecting the presence or absence of a polypeptide or nucleic acid of the invention in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting a polypeptide or nucleic acid (e.g., mRNA, genomic DNA) of the invention such that the presence of a polypeptide or nucleic acid of the invention is detected

35   in the biological sample. A preferred agent for detecting mRNA or genomic DNA encoding a polypeptide of the invention is a labeled nucleic acid probe capable of hybridizing to

mRNA or genomic DNA encoding a polypeptide of the invention. The nucleic acid probe can be, for example, a full-length cDNA, such as the nucleic acid of SEQ ID NO:1, 8, 11, 14, 17 or 20, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent

5   conditions to a mRNA or genomic DNA encoding a polypeptide of the invention. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting a polypeptide of the invention is an antibody capable of binding to a polypeptide of the invention, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a

10   fragment thereof (e.g., Fab or F(ab')$_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a

15   fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect mRNA, protein, or genomic DNA in a biological sample *in*

20   *vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of a polypeptide of the invention include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of genomic DNA include Southern hybridizations. Furthermore, *in vivo*

25   techniques for detection of a polypeptide of the invention include introducing into a subject a labeled antibody directed against the polypeptide. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test

30   subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent

35   capable of detecting a polypeptide of the invention or mRNA or genomic DNA encoding a polypeptide of the invention, such that the presence of the polypeptide or mRNA or

genomic DNA encoding the polypeptide is detected in the biological sample, and
comparing the presence of the polypeptide or mRNA or genomic DNA encoding the
polypeptide in the control sample with the presence of the polypeptide or mRNA or
genomic DNA encoding the polypeptide in the test sample.

5   The invention also encompasses kits for detecting the presence of a polypeptide or
nucleic acid of the invention in a biological sample (a test sample). Such kits can be used to
determine if a subject is suffering from or is at increased risk of developing a disorder
associated with aberrant expression of a polypeptide of the invention (e.g., a proliferative
disorder, e.g., psoriasis or cancer). For example, the kit can comprise a labeled compound
10 or agent capable of detecting the polypeptide or mRNA encoding the polypeptide in a
biological sample and means for determining the amount of the polypeptide or mRNA in
the sample (e.g., an antibody which binds the polypeptide or an oligonucleotide probe
which binds to DNA or mRNA encoding the polypeptide). Kits may also include
instructions for observing that the tested subject is suffering from or is at risk of developing
15 a disorder associated with aberrant expression of the polypeptide if the amount of the
polypeptide or mRNA encoding the polypeptide is above or below a normal level.

           For antibody-based kits, the kit may comprise, for example: (1) a first antibody (e.g.,
attached to a solid support) which binds to a polypeptide of the invention; and, optionally,
(2) a second, different antibody which binds to either the polypeptide or the first antibody
20 and is conjugated to a detectable agent.

           For oligonucleotide-based kits, the kit may comprise, for example: (1) an
oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic
acid sequence encoding a polypeptide of the invention or (2) a pair of primers useful for
amplifying a nucleic acid molecule encoding a polypeptide of the invention. The kit may
25 also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit
may also comprise components necessary for detecting the detectable agent (e.g., an
enzyme or a substrate). The kit may also contain a control sample or a series of control
samples which can be assayed and compared to the test sample contained. Each component
of the kit is usually enclosed within an individual container and all of the various containers
30 are within a single package along with instructions for observing whether the tested subject
is suffering from or is at risk of developing a disorder associated with aberrant expression of
the polypeptide.

           2.   Prognostic Assays
35 ·   The methods described herein can furthermore be utilized as diagnostic or
prognostic assays to identify subjects having or at risk of developing a disease or disorder

associated with aberrant expression or activity of a polypeptide of the invention. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with aberrant expression or activity of a polypeptide of the invention,

5   e.g., a proliferative disorder, e.g., psoriasis or cancer, or an angiogenic disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing such a disease or disorder. Thus, the present invention provides a method in which a test sample is obtained from a subject and a polypeptide or nucleic acid (e.g., mRNA, genomic DNA) of the invention is detected, wherein the presence of the

10   polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant expression or activity of the polypeptide. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine

15   whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, such methods can be used to determine whether a subject can be effectively treated with a specific agent or class of agents (e.g., agents of a type which

20   decrease activity of the polypeptide). Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant expression or activity of a polypeptide of the invention in which a test sample is obtained and the polypeptide or nucleic acid encoding the polypeptide is detected (e.g., wherein the presence of the polypeptide or nucleic acid is diagnostic for a

25   subject that can be administered the agent to treat a disorder associated with aberrant expression or activity of the polypeptide).

The methods of the invention can also be used to detect genetic lesions or mutations in a gene of the invention, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized aberrant expression or activity of a polypeptide of the invention.

30   In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding the polypeptide of the invention, or the mis-expression of the gene encoding the polypeptide of the invention. For example, such genetic lesions or mutations can be detected by ascertaining the existence of at least

35   · one of: 1) a deletion of one or more nucleotides from the gene; 2) an addition of one or more nucleotides to the gene; 3) a substitution of one or more nucleotides of the gene; 4) a

chromosomal rearrangement of the gene; 5) an alteration in the level of a messenger RNA transcript of the gene; 6) an aberrant modification of the gene, such as of the methylation pattern of the genomic DNA; 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; 8) a non-wild type level of a the protein encoded by

5   the gene; 9) an allelic loss of the gene; and 10) an inappropriate post-translational modification of the protein encoded by the gene. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a gene.

In certain embodiments, detection of the lesion involves the use of a probe/primer in

10   a polymerase chain reaction (PCR) (*see, e.g.,* U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.,* Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in a gene (*see, e.g.,* Abravaya et al. (1995) *Nucleic Acids Res.*

15   23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to the selected gene under conditions such that hybridization and amplification of the gene (if present) occurs, and detecting the presence or absence of an amplification product, or

20   detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication

25   (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) *Bio/Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially

30   useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a selected gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction

35 · endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates

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mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations can be identified by hybridizing a sample

5   and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin et al. (1996) *Human Mutation* 7:244-255; Kozal et al. (1996) *Nature Medicine* 2:753-759). For example, genetic mutations can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin et al., *supra*. Briefly, a first hybridization array of probes can be used to scan

10   through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of

15   parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the selected gene and detect mutations by comparing the sequence of the sample nucleic acids with the corresponding wild-type (control)

20   sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Bio/Techniques* 19:448), including sequencing by mass spectrometry (*see, e.g.,* PCT

25   Publication No. WO 94/16101; Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in a selected gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the

30   technique of mismatch cleavage entails providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. RNA/DNA duplexes can be

35   treated with RNase to digest mismatched regions, and DNA/DNA hybrids can be treated with S1 nuclease to digest mismatched regions.

In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.,*

5   Cotton et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called DNA

10 mismatch repair enzymes) in defined systems for detecting and mapping point mutations in cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a selected sequence, e.g., a wild-type sequence, is

15 hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.,* U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in genes. For example, single strand conformation polymorphism (SSCP) may

20 be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2766; *see also* Cotton (1993) *Mutat. Res.* 285:125-144; Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies

25 according to sequence, and the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double

30 stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet.* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) *Nature* 313:495). When DGGE is

35 · used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a `GC clamp of approximately 40 bp of high-melting GC-

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rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not
5   limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) *Nature* 324:163); Saiki et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele specific
10   oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides
15   used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel
20   restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a
25   specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a gene
30   encoding a polypeptide of the invention. Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which the polypeptide of the invention is expressed may be utilized in the prognostic assays described herein.

3.   Pharmacogenomics
35 ·   Agents, or modulators which have a stimulatory or inhibitory effect on activity or expression of a polypeptide of the invention as identified by a screening assay described

herein can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant activity of the polypeptide. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual

5 may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used

10 to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of a polypeptide of the invention, expression of a nucleic acid of the invention, or mutation content of a gene of the invention in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the

15 response to drugs due to altered drug disposition and abnormal action in affected persons. *See, e.g.,* Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body are referred to as "altered drug action." Genetic conditions transmitted as single factors altering the way the body acts on drugs are

20 referred to as "altered drug metabolism". These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

25 As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response

30 and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor

35 · metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active

therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due

5   to CYP2D6 gene amplification.

Thus, the activity of a polypeptide of the invention, expression of a nucleic acid encoding the polypeptide, or mutation content of a gene encoding the polypeptide in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used

10   to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a modulator of activity or expression of the polypeptide, such as a modulator identified by

15   one of the exemplary screening assays described herein.

4.   Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of a polypeptide of the invention (e.g., the ability to modulate aberrant cell

20   proliferation chemotaxis, and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein, to increase gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting decreased gene expression, protein levels, or protein activity. Alternatively, the effectiveness of an

25   agent, as determined by a screening assay, to decrease gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting increased gene expression, protein levels, or protein activity. In such clinical trials, expression or activity of a polypeptide of the invention and preferably, that of other polypeptide that have been implicated in for example, a cellular proliferation disorder, can be used as a marker of the

30   immune responsiveness of a particular cell.

For example, and not by way of limitation, genes, including those of the invention, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates activity or expression of a polypeptide of the invention (e.g., as identified in a screening assay described herein) can be identified. Thus, to study the effect

35   ·of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of a gene of the

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invention and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of a gene of the

5   invention or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring

10   the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of the polypeptide or nucleic acid of the invention in the preadministration sample;

15   (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level the of the polypeptide or nucleic acid of the invention in the post-administration samples; (v) comparing the level of the polypeptide or nucleic acid of the invention in the pre-administration sample with the level of the polypeptide or nucleic acid of the invention in the post-administration sample or samples; and (vi) altering the administration of the

20   agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of the polypeptide to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of the polypeptide to lower levels than detected, i.e., to decrease the effectiveness of the agent.

25

C.   Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, disorders

30   characterized by abberant expression or activity of the polypeptides of the invention include proliferative disorders such as psoriasis and cancer. In addition, the polypeptides of the invention can be used to promote hair growth, promote wound healing, as well as other uses described herein.

35 1.   Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant expression or activity of a polypeptide of the invention, by administering to the subject an agent which modulates expression or at least one activity of the polypeptide. Subjects at risk for a disease which is caused or contributed to by aberrant expression or activity of a polypeptide of the invention can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of aberrancy, for example, an agonist or antagonist agent can be used for treating the subject. For example, an antagonist of an ELVIS protein may be used to treat a proliferative disorder, e.g., psoriasis, associated with abberant ELVIS expression or activity. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating expression or activity of a polypeptide of the invention for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of the polypeptide. An agent that modulates activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of the polypeptide, a peptide, a peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of the polypeptide. Examples of such stimulatory agents include the active polypeptide of the invention and a nucleic acid molecule encoding the polypeptide of the invention that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of the polypeptide of the invention. Examples of such inhibitory agents include antisense nucleic acid molecules and antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a polypeptide of the invention. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) expression or activity. In another embodiment, the method involves administering a polypeptide of the invention or a nucleic acid molecule of the invention as therapy to compensate for reduced or aberrant expression or activity of the polypeptide.

Stimulation of activity is desirable in situations in which activity or expression is abnormally low or downregulated and/or in which increased activity is likely to have a beneficial effect, e.g., in wound healing. Conversely, inhibition of activity is desirable in situations in which activity or expression is abnormally high or upregulated and/or in which

5   decreased activity is likely to have a beneficial effect, e.g., in treatment of a proliferative disorder such as psoriasis.

This invention is further illustrated by the following examples which should not be construed as limiting.

All publications, patents and patent applications mentioned in this specification are

10 herein incorporated by reference in to the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Deposit of Clones

15   Clones containing cDNA molecules encoding TANGO 128, TANGO 140-1, TANGO 140-2, TANGO 197 and TANGO 239 were deposited with the American Type Culture Collection (Manassas, VA) as composite deposits.

Clones encoding TANGO 128, TANGO 140-1, TANGO 140-2, TANGO 197 and TANGO 239 were deposited on November 20, 1998 with the American Type Culture

20 Collection under Accession Number ATCC 98999, (also referred to herein as mix EpDHMix1) from which each clone comprising a particular cDNA clone is obtainable. This deposit is a mixture of five strains, each carrying one recombinant plasmid harboring a particular cDNA clone. To distinguish the strains and isolate a strain harboring a particular cDNA clone, one can first streak out an aliquot of the mixture to single colonies on nutrient

25 medium (e.g., LB plates) supplemented with 100μg/ml ampicillin, grow single colonies, and then extract the plasmid DNA using a standard minipreparation procedure. Next, one can digest a sample of the DNA minipreparation with a combination of the restriction enzymes Sal I and Not I and resolve the resultant products on a 0.8% agarose gel using standard DNA electrophoresis conditions. The digest will liberate fragments as follows:

30   TANGO 128 (EpDH237) 2.8 kb and 4.3 kb
            TANGO 140-1 (EpDH137) 1.6 kb and 3.0 kb
            TANGO 140-2 (EpDH185) 3.4 kb and 4.3 kb
            TANGO 197 (EpDH213) 2.3 kb and 3.0 kb
            TANGO 239 (EpDH233) 3.0 kb and 3.4 kb

35   The identity of the strains can be inferred from the fragments liberated.